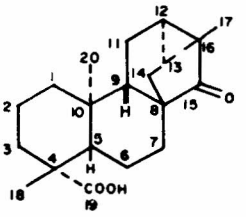
Indian Journal of Chemistry
Vol. 37B, February 1998, pp. 187 - 189

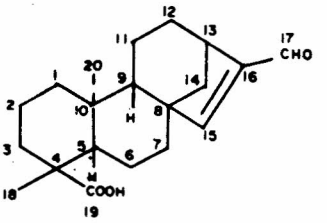
Novel kaurane and trachylobane diterpenes from *Xylopia althiopica* (Dunal) A Rich (Annonaceae)

Edet M Anam
Chemistry Department
University of Calabar
PMB1115, Calabar, CRS, Nigeria

Received 7 February 1997; accepted (revised) 8 January 1998



1



2

Novel diterpenes, 15-oxo-(-)trachyloban-19-oic acid **1** and (-)-kaur-15-en-17-al-19-oic acid **2** have been isolated from the root extract of *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae).

Previous phytochemical studies of *X. aethopica* led to the isolation of kaurane diterpenes from the fruits[1-3] and kaurane, kolavane and trachylobane diterpenes from the stem bark[4]. Methanol extract of the roots of *X. aethiopica* has yielded two new diterpenes 15-oxo-(-)-trachyloban-19-oic acid **1** and (-)-kaur-15-en-17-al-19-oic acid **2**. *n*-Hexane extract of the plant yielded known diterpenes.

Results and Discussion

Compound 1. Mol. formula $C_{20}H_{28}H_3$ was determined by HREIMS and ^{13}C NMR spectroscopy. IR spectrum showed peaks at 1720 (C=O), 3374 and 1692 (-COOH) cm^{-1}. ^1H NMR spectrum showed three methyl singlets at δ 0.98 (3H, s, H-20, δ_c 28.8), 1.21 (3H, s, H-17, δ_c 12.9) and 1.23 (3H, s, H-18 δ_c 28.8). In addition to extensively overlapping methylene and methine signals, the only other discrete signals in the ^1H NMR spectrum were at δ0.83 (1H, dt, *J*=12.7, 4.0 Hz, H-1, δ_c 39.4), 1.52 (1H, br, d, *J*=12.7Hz, H-1, δ_c 39.4), 2.13 (1H, br, d, *J*=12.9Hz, H-3, δ_c 37.6) and 2.44 (1H, d, *J*=11.9Hz, H-13, δ_c 30.3). This suggested that the ketone carbonyl and carboxylic acid were the only functional groups present in **1** which was supported by the signals at δ182.9 (s, C-19) and 218.5 (s, C-15) in its ^{13}C NMR spectrum. The DEPT spectrum indicated the presence of three methyl groups, seven methylenes, four methines and six quaternary carbons. By assuming a trachylobane skeleton and by comparison with carbon assignments of known trachylobane diterpenes[5,6] it was possible to assign the carbonyl group to C-19 and make assignments for C-1-C-6, C-9-C-11 and C-18. These assignments were further supported by HMBC (Heteronuclear Multiple Quantum Bond Connectivity) spectrum long-range connectivity correlations between δ_H1.23 and δ_c 37.6; 43.7 (s, C-4) and 55.4 (d, C-5) and between signals at δ_H 18.5 (t, C-2), 39.2 (s, C-10) and 54.6 (d, C-9). In addition an HMBC correlation between the signal at δ_H 0.83 and δ_c 12.8 allowed the assignment of the C-20 methyl group. The remaining methyl group could therefore be assigned to C-17. This was supported by an HMBC correlation between signals at δ_H 1.21 and δ_c 30.3, 33.3 (s, C-16) which allowed the assignment of the remaining quaternary carbon (C-8, s, δ_c 46.5), 34.2 (d, C-12) and 218.5 positioning the ketone carbonyl at C-15. These facts confirmed **1** to be 15-oxo-(-)-trachyloban-19-oic acid, a compound structurally similar to 15-hydroxy-(-)-trachyloban-19-oic acid[4]. The assignments of the methine carbons C-12 and C-13 were made possible by an HMBC correlation between signals at δ_H 2.44 and δ_c 46.5, 30.4, 33.3 and 218.5 and a ^1H-^1H COSY correlation between δ2.44 and 1.66 (1H, m) which in conjunction with its HETCOR spectrum can be assigned as H-12. It is evident that the carbonyl function at C-15 results in considerable deshiedling of the cyclopropane system (H12 : H13) of **1** (cf. Table I).

Compound 2. Mol. formula $C_{20}H_{28}O_3$ was determined by HREIMS and ^{13}C NMR

Table I—^{1}H and ^{13}C NMR data of 1 and 2

	1					2				
Proton	Chem. Shift ·0.83	Mult.	J=Hz	δ_c †	HMBC**	Chem Shift	Mult.	J=Hz	δ_c †	HMBC**
H-1	1.52	(1H,dt)	12.7,4.0	39.4(t)		0.83	(1H,dt)	12.6,4.0	40.6(t)	H-20
	2.13	(1H,br,d)	12.7							
H-2				18.5(t)	H-1				19.0(t)	
H-3		(1H,br,d)	12.9	37.6(t)	H-18				37.8(t)	H-18
H-4				43.7(s)	H-18				43.9(s)	H-18
H-5				55.4(d)	H-18				56.5(d)	H-18,H-20
H-6				19.9(t)		2.18	(2H,d)	11.0*	23.3(t)	
H-7				30.5(t)					43.0(t)	
H-8				46.5(s)	H-13				50.9(s)	H-15
H-9	1.40	(1H,m)		56.6(d)	H-1,H-12				45.9(d)	H-20
H-10				39.2(s)	H-1,H-9,H-20				40.0(s)	
H-11				18.6(t)	H-9				18.7(t)	
H-12	1.66	(1H,m)		34.2(d)	H-17				25.1(t)	
H-13	2.44	(1H,d)	11.9	30.3(d)	H-12,H-17	2.18	(2H,d)	11.0*	37.8(d)	H-17
H-14				30.4(t)	H-9,H-13	3.03	(1H,d)	3.0	38.2(t)	H-15
H-15				218.5(s)	H-9,H-13,H-17	6.58	(2H,s)		161.5(d)	H-13
H-16				33.3(s)	H-13,H-17				148.7(s)	H-13,H-17
H-17	1.21	(3H,s)		12.9(q)		9.74	(1H,s)		189.8(d)	H-15
H-18	1.23	(3H,s)		28.8(q)		1.26	(3H,s)		28.9(q)	
H-19				182.9(s)					182.6(s)	H-18
H-20	0.98	(3H,s)		12.8(q)	H-1,H-9	1.00	(3H,s)		15.5(s)	

†Multiplicity deduced from a DEPT experiment
†^{1}H-^{13}C Correlation from a HETCOR experiment
**Protons showing long range correlations to indicated carbon
‡^{1}H-^{13}C correlation from a HMQC [^{1}H detected one-bond heteronuclear multiple quantum coherence] experiment in 2.
*Signal composed of two overlapping 1H signals.

spectroscopy. IR spectrum showed peaks at 1695 and 1684 (two C=O groups) and 1652 (C=C) cm^{-1} suggesting one of the carbonyls to be conjugated. The ^{1}HNMR and ^{13}C NMR spectra supported the presence of a conjugated aldehyde system with signals at δ_H 6.58 (1H, s, H-15, δ_c 161.5), 9.74 (1H, s, H-17, δ_c 189.5) and 148.7 (s, C-16). Two methyl singlets were observed in the ^{1}H NMR spectrum at δ1.00 (3H, s, H-20, δ_c 15.5) and 1.26 (3H, s, H-18, δ_c 28.9). Other signals included δ0.83 (1H, dt, *J*=12.6, 4.0 Hz, H-1, δ_c 40.6), 2.18 (2H, d, *J*=11.0 Hz, H-7 and H-13) and 3.03 (1H, d, *J*=3.0 Hz, H-14). The DEPT spectrum indicated the presence of two methyl groups, eight methylenes, five methines and five quaternary carbons one of which is a carboxylic acid carbon (s, 182.6). Compound **2** is assumed to have a kaurane skeleton and comparison with carbon assignments of known kaurane diterpenes[6] allowed the assignment of -COOH group at C-19 and the assignments of C-1-C-6, C-10-C-12, C-18 and C-20. These assignments were supported by HMBC correlations between signals at δ_H1.26 and δ_c 37.8 (t, C-3), 43.9 (s, C-4), 56.5 (d, C-5) and 182.6 and between signals at δ_H 1.00 and δ_c 40.6, 45.9 (d, H-9) and 56.5 (cf. Table I).

Comparison with the spectral data of known aldehyde bearing kauranes[6,7], placed the conjugated aldehyde system on the D-ring with the double bond at C-15 (16) and the aldehyde group at C-17 establishing the structure of **2** as (-)-Kaur-15-en-17-al-19-oic-acid. HMBC correlations between signals at d_H 6.58 and δ_c 38.2 (d, C-14) 50.8 (s, H-15) and 189.8 and between signals at δ_H 9.74 and δ_c 37.8 (d, C-13) and 148.7 (s, C-16) made these assignments possible and supported the structural identity of compound **2**. C-14 signals were assigned on the basis of a HETCOR correlation between signals at δ_H 3.03 and δ_c 38.2 and this made the remaining assignments of C-7, C-8 and C-9 as δ_c 44.0 (t), 50.9(s) and 45.9(d) respectively possible.

Experimental Section

The plant material was collected in January 1996 from the riverine area of Odukpani LGA, CRS, Nigeria and was authenticated by the Botany Division of the Department of Biological Sciences, University of Calabar, Calabar. A voucher specimen documenting the collection is deposited in the Herbarium of the University of Calabar.

Optical rotation $[\alpha]_D$ were measured on a Perkin-Elmer 241 polarimeter. IR spectra were run on a Perkin-Elmer double beam spectrometer 580B and KBr pellets. ^1H (at 270 or 400 MHz) and ^{13}C NMR (at 100.57 MHz) spectra were run on Bruker AMX 400 in $CDCl_3$, solutions using TMS as internal standard. HETCOR and ^{13}C-^1H correlations spectra were performed with the use of Bruker microprogrammes. EIMS at 70eV on a Varian MAT700 spectrometer. 2DNMR was performed at 100.57 MHz with TMS as internal standard. Standard Bruker Pulse sequences were used for both direct and long range heteronuclear correlation experiments.

Extraction and isolation of diterpenes. Oven dried ground roots (500 g) of *X. aethiopica* were soxhlet extracted first with *n*-Hexane and then with MeOH for 24 hr each. The conc. MeOH extract (32.50 g) was partitioned between *n*-BuOH and water (1:1). The BuOH portion was further partitioned between $CHCl_3$ and 60% aq. MeOH (1:1). The 80% aq MeOH soluble fraction was then subjected to adsorption chromatography on Sephadex LH-20[8] eluting with *n*-Hexane-CH_2Cl_2 (1:1) followed by CH_2Cl_2 and finally MeOH. The CH_2Cl_2 eluate was further chromatographed on Sephadex LH-20 eluting with *n*-Hexane-CH_2Cl_2 (4:1); *n*-Hexane-CH_2Cl_2 (1:1), CH_2Cl_2 - Me_2CO (1:1) and finally MeOH. The *n*-Hexane-CH_2Cl_2 (1:1) and the CH_2Cl_2 portions were then pooled and subjected to PTLC on silica gel GF_{254} (1 mm thick) using CH_2Cl_2 - i-PrOH (19:1) as eluent. A broad strongly UV absorbing band was collected at R_f 0.40 and resubjected to PTLC using CH_2Cl_2-i-PrOH (99:1) as eluent. Two bands 1 and 2 were separated. Band 1 gave 45 mg of a known compound, sitosterol glucoside. Band 2 which indicated the presence of two compounds were subjected to reversed phase HPLC using 72% aq. MeOH as eluent at a flow rate of 1.5 mL min^{-1}. This yielded **1** (R_t 5.0 min; 9.0 mg) and **2** (R_t 7.5 min; 1.85 mg).

Compound 1. Pale yellow gum; $[\alpha]_0$ -83.5° ($CHCl_3$, c 1.2); IR: 3374, 2918, 2849, 1720, 1692, 1462, 1444 and 755 cm^{-1}; HREIMS m/z (rel. int.) [M^+] 316.2026 (100) [$C_{20}H_{28}O_3$ requires 316.2038]; ^1H NMR ($CDCl_3$, 270 MHz) and ^{13}C NMR, ($CDCl_3$, 100.57 MHz) data are given in Table I.

Compound 2. Pale yellow gum; $[\alpha]_0$ - 21.9° ($CHCl_3$, c 1.2); IR : 3400, 2936, 2869, 1695, 1684, 1652, 1462, 1444, 1368, 1259, 1172 and 756 cm^{-1}: HREIMS m/z (rel. int.) [M^+] 316.2049 (50) [$C_{20}H_{28}O_3$ requires 316.2083]; ^1H NMR ($CDCl_3$, 270 MHz) and ^{13}C NMR ($CDCl_3$, 100.57 MHz) data are given in Table I.

Acknowledgement

The author is grateful to the Department of Chemistry, University of London for running the spectra of the compounds.

References

1 Ekong D E U & Ogan A U, *J Chem Soc (C)*, **1968**, 311
2 Ekong D E U, Olagbemi E O & Odutola F A, *Phytochemistry*, 8, **1969**, 1053.
3 Hasan C M, Haeley T M & Waterman P G, *Phytochemistry*, 21, **1982**, 1365.
4 Faulkner D F, Lebby V & Waterman P G, *Planta Med*, 53, **1985**, 354.
5 Arnone A, Mondelli R & St Pyrek J, *Org Magn Res*, 12, **1979**, 429.
6 Rahman A & Ahmad V U (eds), *^{13}C NMR of Natural Products; Diterpenes)*, Vol 2, (Plenum Press, New York), **1992.**
7 Fernandez C, Fraga B M & Hernandez M G, *Phytochemistry*, 25, **1986**, 2573.
8 Cardellina J, *J Nat Prod*, 46, **1983**, 196.